The best defence

Professor Roger Innes and his team of diverse specialists are uncovering key insights into both the plant and animal immune system

Image courtesy of Indiana University

Could you explain the objectives of your NIH projects, and the questions about plant disease resistance underpinning them?

Our projects approach the question of how plants defend themselves against pathogens from two opposing directions. The first focuses on the molecular mechanisms plants use to detect pathogens and activate defences, while the second seeks to understand how the defence system is normally kept off in the absence of pathogens. Inappropriate activation of defence responses – in plants as in animals - is highly deleterious, so defence activation is under complex positive and negative regulation, which my NIH-funded projects seek to unravel.

What methods have you adopted to investigate how plants detect pathogens?

We use molecular biology techniques to alter the DNA sequence of genes known to mediate pathogen recognition, which we call Resistance (*R*) genes. Then, we express these mutated *R* genes transiently in leaves, where we test for interactions of *R* proteins with other proteins, their ability to activate Programmed Cell Death (PCD ; an important component of the defence response), and determine their subcellular location using fluorescence microscopy. This allows us to determine which domains of *R* proteins are required to activate PCD, and to identify additional plant proteins required for activation of defence responses.

Can you alter the disease resistance of a plant by modifying *R* genes, or transferring *R* genes from one plant species to another?

Yes, it is now fairly straightforward to modify genes and put them back into a plant, or to transfer genes from one plant species to another, using recombinant DNA techniques. This makes it feasible to identify a useful *R* gene in a wild plant species and transfer the gene into a crop plant. A really nice example of this is a gene from a wild potato relative that confers resistance to late blight in potato, the disease that caused the Irish potato famine.

What effect will this technique have on the virulence of plant diseases and could this approach preclude pathogens from attacking?

R genes usually confer resistance to just a few strains of a given pathogen species, so to confer resistance to the majority or complete resistance to a disease such as potato late blight (*Phytophthora infestans*), can require 'stacking' of multiple resistance genes. Recent research has discovered *R* genes from wild relatives of potato that could collectively confer resistance to virtually all known late blight strains. Using recombinant DNA techniques, we could put these genes into commercial potato varieties, obviating the need for huge amounts of pesticides: highly desirable from both a food consumption and environmental perspective.

Further to the above, are there any dangers in altering signaling transducing mechanisms that regulate gene expression?

R proteins function at the top of a signal transduction cascade, and are normally kept in the off state in which they exert little effect on the plant. However, if activated they lead to massive defence responses including PCD. Sometimes transferring *R* proteins from one plant species to another can lead to inappropriate activation, turning on unneeded defence responses that in turn cause dwarfing and dead patches - understanding how to control this is part of my research. Similarly, mutations in signalling steps downstream of *R* proteins can cause inappropriate defence activation: understanding this will enable us to manipulate defence pathways more intelligently and ensure only necessary defence responses are activated.

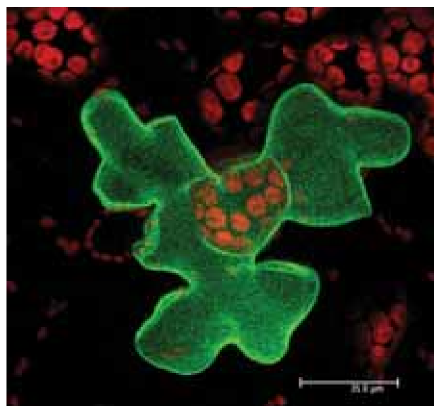
What have been your main findings to date, and what questions remain unanswered?

Most significantly that many - perhaps most - *R* proteins detect pathogen proteins

indirectly by detecting modification of the targets of pathogen virulence proteins. This has provided us with an understanding of how single receptors can detect multiple pathogens, including unrelated ones such as nematodes and insects. It also gave us better understanding of how plants keep up in the 'arms race' with pathogens that have much shorter generation times. For a pathogen to evade detection, it would require a new way of causing disease. It appears that a similar indirect mechanism may also apply to human immune system proteins such as NOD2. The biggest unanswered question is how *R* proteins activate programmed cell death. We assume that the activated protein interacts with other proteins, causing calcium channels to open and an increase in production of activated oxygen species - but what those proteins are is currently unknown.

What causes pathogens to escape recognition and cause disease in plants?

Usually it has mutated and lost expression of the specific virulence protein detected by the plant *R* proteins. One would expect a loss in pathogen virulence after such a mutation, but this is not always the case because pathogens produce dozens of virulence proteins with overlapping functions. Often, losing just one does not compromise virulence because those remaining suffice. Plants combat this by evolving lots of *R* proteins that 'guard' the targets of many different pathogen virulence proteins. Comparing the genome sequences of individuals of the same plant species, there is a huge variety of *R* proteins, evolving faster than any other plant gene family, indicative of an arms race.

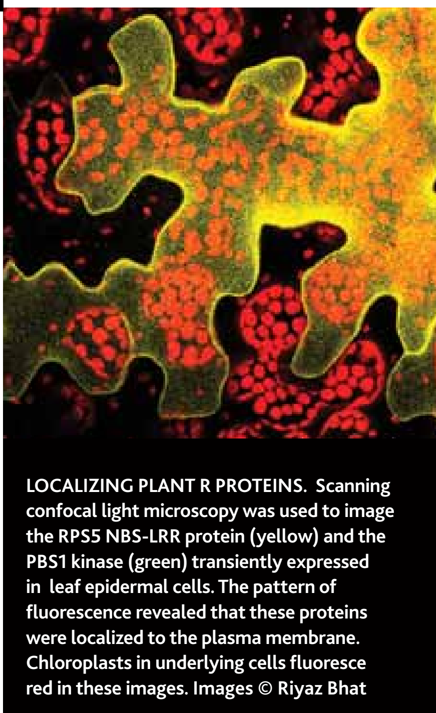


In regards to your second project, how does the EDR1 protein kinase of *Arabidopsis*, and mutations of it, relate to disease resistance and PCD?

Loss of EDR1 function leads to a 'paranoid' phenotype whereby even minor biotic or abiotic stresses (e.g. a normally virulent powdery mildew strain, or drought) activate defence responses and PCD. Although the reasons why are not yet clear, recent data indicate that *edr1* mutants have a defect in a cellular recycling system called autophagy. Autophagy takes cellular components such as protein complexes, organelles, and bulk cytoplasm, envelopes them in a membranous vesicle, then delivers them to the plant vacuole where they are broken down into their constituent building blocks for synthesizing new proteins and organelles. Autophagy is likely critical to removal of damaged mitochondria and chloroplasts, which are major sources of reactive oxygen species and free radicals. If autophagy is not working, it is likely that the increase in these compounds leads to rapid cell death during times of stress.

In light of your main findings to date, which direction do you see the research taking?

Two key findings have been that *edr1* mutants are likely defective in autophagy and that all edr1-associated phenotypes can be suppressed by specific mutations in another protein known as KEEP ON GOING (KEG). The latter is important as it links *edr1* phenotypes to protein degradation because KEG functions to target specific proteins for breakdown. We believe *keg* mutants that suppress *edr1* enhance degradation of specific targets of KEG. The critical question is: what are these target proteins? Identifying these targets is currently a focus in the lab. Ultimately, we expect to provide exciting new insights into the links between autophagy and defence responses in plants. This is a hot area in animal immunology, just starting to heat up in plant research, and we anticipate that both fields will inform each other in the years to come.



LOCALIZING PLANT R PROTEINS. Scanning confocal light microscopy was used to image the RPS5 NBS-LRR protein (yellow) and the PBS1 kinase (green) transiently expressed in leaf epidermal cells. The pattern of fluorescence revealed that these proteins were localized to the plasma membrane. Chloroplasts in underlying cells fluoresce red in these images. Images © Riyaz Bhat

A Real Alternative to Pesticides

Led by Professor Roger Innes, a pioneering team at Indiana University's NIH-funded Innes Lab is sowing the seeds for tomorrow's disease-resistant crops

IN THE BATTLE to defend commercial crops against disease, producers in the U.S. alone use over two million tonnes of pesticide annually; this carries a substantial burden, both financially, and, some would say, in health terms. But by understanding a plant's disease resistance mechanisms at the molecular level, resistant crops that require less energy and pesticides to produce are just around the corner.

Professor Innes' work has taken two approaches: firstly, looking at how plants recognise pathogens; and secondly, in studying what happens when a plant's defences are activated in error. By using a model system *Arabidopsis thaliana* – preferred in plant molecular biology – the team was able to explore how the plant's RPS5 protein mediates recognition of the AvrPphB protein secreted by the bacterial pathogen *Pseudomonas syringae*, which causes bacterial speck disease in many plant species.

As Innes explains, a comprehensive understanding of plant defences requires a multi-focused approach: "We need to know which proteins are involved, where they are in the cell, how they interact with each other, and how they change shape and location upon detection of a pathogen."

GREAT POTENTIAL FOR NBS-LRR PROTEINS

The Innes Lab's early classical genetic research contributed to the discovery of a large family of plant proteins that act as intracellular receptors to detect the presence of pathogen proteins. These plant proteins share a similar structure characterised by a nucleotide binding site (NBS; specifically a binding site for the nucleotide ATP) and leucine-rich repeats (LRR).

The team's research has shown that many NBS-LRR proteins detect pathogens indirectly by detecting pathogen-induced modification of host proteins. This throws up many other questions still to be addressed, not least the role of the ATP binding and the LRR domain in this process, as well as how the NBS-LRR proteins detect pathogen-induced modifications of

SYMPTOMS OF A 'PARANOID' MUTANT. The *enhanced disease resistance 1`(edr1)* mutant plant forms patches of dead cells on non-inoculated leaves in response to infection of other leaves on the same plant. Wild-type plants (Col-0) do not form such lesions, and neither do *edr1* plants containing mutations in a second gene called *keg* (for Keep on Going). The suppressor 69 plant contains both the *edr1* and *keg-4* mutations.

Photograph © Yangnan Gu

host proteins, and identification of the proteins that function downstream of NBS-LRR proteins to turn on defence responses.

Innes points out that this work has applications in both agriculture and human health. Significantly, similar proteins to NBS-LRR proteins have been discovered in humans – such as the intracellular protein NOD2 – mutations in which appear to be one of the main causes of the autoimmune disorder, Crohn's disease.

Studying these proteins present technical challenges as they exist at extremely low levels in the cell under normal conditions, and consequently are nearly impossible to see by light microscopy. In addition, they have proven impossible to express in a functional form in standard laboratory expression systems such as *E. coli*. Progress in understanding how NBS-LRR proteins function will require purification of protein from plant extracts, but Innes is optimistic in regard to the potential of this approach: "By pursuing purification from large scale leaf extracts, we will soon have sufficient quantities of sufficiently pure protein for our analyses", he states.

'PARANOID' MUTANTS

Another aspect of the Innes Lab's research concentrates on why certain mutations cause erroneous activation of defence responses, answers to which are crucial to development of crop varieties with enhanced disease resistance. Of particular interest are 'paranoid' mutants, whose massive defensive responses to any kind of biotic or abiotic stress, leads to programmed cell death (PCD). When properly working, PCD can be a critical component of animal and plant immune systems, stopping the flow of nutrients and water to the pathogen, while adjacent cells pump out antimicrobial compounds to break down the pathogen cell walls. However, when PCD misfires, the entire plant can die: "'Paranoid mutants' have lost the function of a negative regulator that would normally keep the PCD confined to a few cells," explains Innes. By understanding these negative regulators, a better understanding should be reached not only of PCD and how it is switched on, but of cellular homeostasis in general.

INTERNATIONAL AND COLLABORATIVE

The multidisciplinary nature of the Innes Lab, as well as its strong collaborative links with other departments, is a driving force behind it. Amongst the broad range of team expertise there are outstanding talents in fluorescence microscopy, protein purification and biochemistry. The lab also benefits from the skill of colleagues in the chemistry department - for protein identification using mass spectrometry, and has collaborated extensively with other universities on comparative genomic analyses, particularly in phylogenetics.

For the last 18 years, the team has comprised at least 50 per cent international scientists, including those from England, India, China, Poland, Africa - as well as the U.S. Innes believes this internationalism has been highly beneficial: "I very much enjoy the multi-cultural atmosphere that international scientists bring to the lab, and certainly," he explains, "my lab would not have been nearly as productive without it."

THE FUTURE

While GM crops have had their critics, Innes is keen to emphasise that over the last decade, they have already enjoyed widespread production in the U.S. without adverse health or environmental impacts; clearly, their benefits are too substantial to ignore.

The Innes Lab's work with NBS-LRR proteins is already being applied in molecular breeding programmes, and this bodes well for future progress. Indeed, Innes is confident that NBS-LRR transference between plant species using recombinant DNA methods is imminent: "We will probably see this approach being applied in commercial crops within the next five years."

INTELLIGENCE

GENETIC ANALYSIS OF DISEASE RESISTANCE IN *ARABIDOPSIS THALIANA*

PROGRAMMED CELL DEATH AND DISEASE RESISTANCE IN *ARABIDOPSIS*

FUNDING: NIH

OBJECTIVES

Research in the Innes Lab addresses two fundamental questions in plant biology: How do plants detect pathogens, and how does pathogen detection lead to activation of defenses? This research will enable development of disease resistant crop plants that will require less energy and less pesticides to produce.

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DR ROGER INNES is Professor and Chair of the Department of Biology at Indiana University. He earned his PhD in Molecular Biology at the University of Colorado, and BA in Biology at Humboldt State University in California. He has made seminal contributions to our understanding of disease resistance mechanisms in plants.

Representative publications from the Innes lab include:

Ade J, DeYoung BJ, Golstein C, Innes RW (2007) Indirect activation of a plant nucleotide binding site-leucine-rich repeat protein by a bacterial protease. *Proc Natl Acad Sci* USA 104: 2531-2536

Wawrzynska A, Christiansen KM, Lan Y, Rodibaugh NL, Innes RW (2008) Powdery mildew resistance conferred by loss of the ENHANCED DISEASE RESISTANCE1 protein kinase is suppressed by a missense mutation in KEEP ON GOING, a regulator of abscisic acid signaling. *Plant Physiol* 148: 1510-1522

Image courtesy of Indiana University